**D:\SFRP4\写作\Supplementary materials\Supplementary Figure 1.tifSupplementary Fig. 1**

**Supplementary Fig. 1.** The genome profiles of different TNM stages of gastric cancer from GEO dataset (GSE27342).

**D:\SFRP4\写作\Supplementary materials\Supplementary Figure 2.tifSupplementary Fig. 2**

**Supplementary Fig. 2.** The expression of SFRPs from online datasets. (A)The expression of SFRP1, SFRP2, and SFRP5 in all cancer types from Oncomine database.(B) A meta-analysis of the expression of SFRP1, SFRP2, and SFRP5 in 12 gastric cancer microarray datasets from Oncomine database. (C) The mRNA level of SFRP1, SFRP2, and SFRP5 in gastric cancer and adjacent normal tissues from TCGA (The Cancer Genome Atlas) dataset. The box plot showed the full range of variation (error bars: min and max) with the line representing median. STAD: Stomach Adenocarcinoma. Statistical significance was determined by Student’s t-test (C).

**Supplementary Fig. 3**

**Supplementary Fig. 3.** Clinical significance of SFRP4 in sections of gastric cancer by immunohistochemical staining. (A-J) The proportion of gastric cancer patients with high or low SFRP4 expression according to age, gender, tumor size, localization, differentiation, Lauren classification, depth of tumor invasion (T stage), lymph node metastasis (N stage), lymphovascular invasion (LVI) and TNM stage. Statistical significance was determined by Pearson’s test or Fisher’s exact test (A-J).

**Supplementary Fig. 4**





**Supplementary Fig. 4.** The prognostic significance of SFRP4 from Zhongshan cohort. (A-K) The association of SFRP4 expression with overall survival was examined by Kaplan-Meier analysis in different subgroups of patients according to T stage (A-D), N stage (E-H) and TNM stage (I-K). Statistical significance was determined by Log-rank test (A-K).

**D:\SFRP4\写作\Supplementary materials\Supplementary Figure 3.tifSupplementary Fig. 5**

**Supplementary Fig. 5.** The prognostic significance of SFRP4 from online datasets. (A) The association of SFRP4 expression with overall survival of gastric cancer by analyzing the data from TCGA dataset. (B) The association of SFRP4 expression with disease-free survival of gastric cancer by analyzing the data from GEO dataset (GSE26253). (C-D) The association of SFRP4 expression with overall survival and disease-free survival of gastric cancer by using online Kaplan-Meier plotter (<http://www.kmplot.com/analysis/index.php?p=service&cancer=gastric>). Statistical significance was determined by Log-rank test (A-D).

**Supplementary Fig. 6**



**Supplementary Fig. 6.** Prognostic model to predict overall survival in patients with gastric cancer. (A) The multivariate analysis of independent prognostic factors was performed by the Cox’s proportional hazards regression mode. (B) ROC analysis of the sensitivity and specificity for the prognosis of 5-year OS by TNM stage/SFRP4 expression model, TNM stage model, and SFRP4 expression model. Delong. Delong. Clarke-Pearson test was used to compare each two ROC curves. HR: hazard ratio; ROC, receiver operating characteristic; AUC, area under the ROC curve; CI, confidence interval. \*\*\**P*<0.001, n.s, non-significant. Statistical significance was determined by Delong. Delong. Clarke-Pearson test (B).

**Supplementary Fig. 7**



**Supplementary Fig. 7.** The colony formation of MGC80-3 and AGS cells treated with recombinant SFRP1 and SFRP4 (upper panel), and quantification of the colonies (lower panel). Error bars indicate mean ± SEM. \*\*\**P*<0.001. r: recombinant. The images were representative of three independent experiments. Statistical significance was determined by One way ANOVA test (B).

**Supplementary Fig. 8**





**Supplementary Fig. 8.** Promoter methylation status of SFRPs in gastric cancer. (A-C) The methylation status of SFRP1, SFRP2, SFRP5, and SFRP4 between normal tissues and gastric cancer tissues were obtained and analyzed from GEO datasets (GSE25869, GSE30601) and TCGA dataset. (D) The methylation status of SFRP4 between normal tissues and gastric cancer tissues were analyzed from TCGA dataset according to TNM stages. The box plot showed the full range of variation (error bars: min and max) with the line representing median. (E-G) The methylation status of SFRP1, SFRP2, SFRP5, and SFRP4 in gastric cancer tissues were obtained and analyzed from GEO datasets (GSE25869, GSE30601) and TCGA dataset. (H) The methylation status of SFRP1, SFRP2, SFRP5, and SFRP4 in gastric cancer tissues were analyzed from TCGA dataset according to TNM stages. Error bars indicate mean ± SEM. (I) The methylation status of SFRP1, SFRP2, SFRP5, and SFRP4 in different gastric cancer cells were obtained and analyzed from GEO dataset (GSE25869). (J) The methylation status of SFRP1, SFRP2, SFRP5, and SFRP4 in 5 gastric cancer specimens from Zhongshan Hospital were determined by quantitative methylation analysis based on Sequenom MassARRAY platform. N: normal; T: tumor; GC: gastric cancer. \*\*\**P*<0.001, \*\**P*<0.01, \**P*<0.05, n.s, non-significant. Statistical significance was determined by Student’s t-test (A-D, H), or One way ANOVA test (E-G, I, J).

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| |  |  |  |  | | --- | --- | --- | --- | | **Supplementary Table 1. Cut-off score determined by ROC curve** | | | | | Cut-off score | Sensitivity | 1-Specificity | Youden index | | 2 | 0.928 | 0.920 | 0.008 | | 3 | 0.830 | 0.671 | 0.159 | | 4 | 0.659 | 0.296 | 0.363 | | 6 | 0.398 | 0.184 | 0.214 | | 8 | 0.261 | 0.132 | 0.129 | | Abbreviations: ROC: receiver operating characteristic. Youden index=Sensitivity-(1-Specificity). | | | | |

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| **Supplementary Table 2. List of PCR Primers** | | |
| Assay | Primer name | Sequence |
| qPCR | WNT2 forward | AGGATGCCAGAGCCCTGATGAA |
| qPCR | WNT2 reverse | AGCCAGCATGTCCTGAGAGTAC |
| qPCR | WNT3 forward | GCGTGTTAGTGTCCAGGGAGTT |
| qPCR | WNT3 reverse | TGAGGTGCATGTGGTCCAGGAT |
| qPCR | WNT4 forward | GCTGGAGAAGTGCGGCTGTGA |
| qPCR | WNT4 reverse | CCACAAACGACTGTGAGAAGGC |
| qPCR | WNT5A forward | TACGAGAGTGCTCGCATCCTCA |
| qPCR | WNT5A reverse | TGTCTTCAGGCTACATGAGCCG |
| qPCR | GSK-3β forward | CCGACTAACACCACTGGAAGCT |
| qPCR | GSK-3β reverse | AGGATGGTAGCCAGAGGTGGAT |
| qPCR | CTNNB1 forward | CACAAGCAGAGTGCTGAAGGTG |
| qPCR | CTNNB1 reverse | GATTCCTGAGAGTCCAAAGACAG |
| qPCR | LEF1 forward | CTACCCATCCTCACTGTCAGTC |
| qPCR | LEF1 reverse | GGATGTTCCTGTTTGACCTGAGG |
| qPCR | CCND1 forward | TCTACACCGACAACTCCATCCG |
| qPCR | CCND1 reverse | TCTGGCATTTTGGAGAGGAAGTG |
| qPCR | PLAUR forward | CCACTCAGAGAAGACCAACAGG |
| qPCR | PLAUR reverse | GTAACGGCTTCGGGAATAGGTG |
| qPCR | MYC forward | CCTGGTGCTCCATGAGGAGAC |
| qPCR | MYC reverse | CAGACTCTGACCTTTTGCCAGG |
| qPCR | JUN forward | CCTTGAAAGCTCAGAACTCGGAG |
| qPCR | JUN reverse | TGCTGCGTTAGCATGAGTTGGC |
| qPCR | CD44 forward | CCAGAAGGAACAGTGGTTTGGC |
| qPCR | CD44 reverse | ACTGTCCTCTGGGCTTGGTGTT |
| qPCR | MMP7 forward | TCGGAGGAGATGCTCACTTCGA |
| qPCR | MMP7 reverse | GGATCAGAGGAATGTCCCATACC |
| qPCR | GAPDH forward | GCCGGTGCTGAGTATGTC |
| qPCR | GAPDH reverse | CTTCTGGGTGGCAGTGAT |
| PCR | SFRP1 forward | aggaagagagGGTAGTTTTATTTTGGGGTTTGGA |
| PCR | SFRP1 reverse | cagtaatacgactcactatagggagaaggctCCAACAACTAAATACCCCTACTCAA |
| PCR | SFRP2 forward | aggaagagagTTTTGTTGGGTTGTTAGGTATGAGT |
| PCR | SFRP2 reverse | cagtaatacgactcactatagggagaaggctCTCCCAACCTACCCATCTTTTATA |
| PCR | SFRP4 forward | aggaagagagTGGAGAGGAATATGGTATTGTTTTT |
| PCR | SFRP4 reverse | cagtaatacgactcactatagggagaaggctAACTACCAAAAATTCTTTTTCTTAATCC |
| PCR | SFRP5 forward | aggaagagagTTTTAGTAGTTTGGTTGGATGGGTA |
| PCR | SFRP5 reverse | cagtaatacgactcactatagggagaaggctATACTACACTACCACAAATTCCCCC |
| Abbreviations: PCR：polymerase chain reaction; qPCR: quantitative real-time PCR; SFRP: Secreted frizzled-related protein. | | |

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| **Supplementary Table 3. Top 20 up-regulated genes in advanced gastric cancer from GSE27342** | | | | | | | |
| Rank | Gene Symbol | Gene Name | LogFC | AveExpr | t | P. value | adj.  P.Val |
| 1 | CST1 | cystatin SN | 2.1715 | 5.995516 | 8.402 | ﹤0.001 | ﹤0.001 |
| 2 | CEACAM6 | carcinoembryonic antigen-related cell adhesion molecule 6 | 1.8480 | 6.564580 | 6.321 | ﹤0.001 | ﹤0.001 |
| 3 | CLDN7 | claudin 7 | 1.8129 | 7.072439 | 8.354 | ﹤0.001 | ﹤0.001 |
| 4 | SFRP4 | secreted frizzled-related protein 4 | 1.7539 | 6.266290 | 7.224 | ﹤0.001 | ﹤0.001 |
| 5 | CLDN4 | claudin 4 | 1.6226 | 6.608403 | 7.912 | ﹤0.001 | ﹤0.001 |
| 6 | MMP1 | matrix metallopeptidase 1 | 1.6157 | 6.265014 | 7.134 | ﹤0.001 | ﹤0.001 |
| 7 | CLDN1 | claudin 1 | 1.5854 | 5.860255 | 9.172 | ﹤0.001 | ﹤0.001 |
| 8 | COL1A1 | collagen, type I, alpha 1 | 1.5587 | 9.296357 | 9.458 | ﹤0.001 | ﹤0.001 |
| 9 | SPP1 | secreted phosphoprotein 1 | 1.5388 | 6.418854 | 7.087 | ﹤0.001 | ﹤0.001 |
| 10 | CDH17 | cadherin 17 | 1.4960 | 5.626618 | 4.965 | ﹤0.001 | ﹤0.001 |
| 11 | DMBT1 | deleted in malignant brain tumors 1 | 1.4132 | 7.079791 | 4.380 | ﹤0.001 | ﹤0.001 |
| 12 | FAP | fibroblast activation protein alpha | 1.3726 | 4.095170 | 7.877 | ﹤0.001 | ﹤0.001 |
| 13 | PLA2G7 | phospholipase A2 group VII | 1.3650 | 6.082009 | 7.807 | ﹤0.001 | ﹤0.001 |
| 14 | SULF1 | sulfatase 1 | 1.3638 | 6.363387 | 8.363 | ﹤0.001 | ﹤0.001 |
| 15 | THY1 | Thy-1 cell surface antigen | 1.3434 | 7.186268 | 8.936 | ﹤0.001 | ﹤0.001 |
| 16 | MMP7 | matrix metallopeptidase 7 | 1.3389 | 6.336472 | 6.055 | ﹤0.001 | ﹤0.001 |
| 17 | TPX2 | TPX2 microtubule nucleation factor | 1.3084 | 5.810727 | 7.966 | ﹤0.001 | ﹤0.001 |
| 18 | TOP2A | DNA topoisomerase II alpha | 1.2763 | 5.282800 | 7.232 | ﹤0.001 | ﹤0.001 |
| 19 | MMP12 | matrix metallopeptidase 12 | 1.2718 | 5.250495 | 4.934 | ﹤0.001 | ﹤0.001 |
| 20 | SERPINB5 | serpin family B member 5 | 1.2436 | 4.631414 | 6.695 | ﹤0.001 | ﹤0.001 |
| Abbreviations: LogFC: Log2 (Fold Change); AveExpr: Average expression of the gene;  t: t-test value; adj.P.Val: adjust. P. value, or FDR (False discovery rate) value. | | | | | | | |